

Sulphydryl sensitivity of syphilitic antibodies and temperature dependence in complement fixation

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Antibodies from syphilitic sera have been studied by ultracentrifugation (Davis, Moore, Kabat, and Harris, 1945; Aho, 1967), paper electrophoresis (Laurell, 1955), immunoelectrophoresis (Nørredam and Clausen, 1963), gel diffusion (Laurell, Oxelius, and Rorsman, 1968), or by DEAE cellulose column chromatography (Laurell and Malmquist, 1961), and gel filtration on Sephadex G-200 (Julian, Logan and Norins, 1969). The immunoglobulin classes in sera of patients with late syphilis have been studied using monospecific fluorescent antisera (Logan, Norins, Atwood, and Miller, 1969). Immunoglobulin sequence has also been studied. Different results were obtained, however, and because these methods are time-consuming only a few sera were examined.

It is generally accepted that the two most important classes of immunoglobulins differ in sedimentation characteristics as well as in their sensitivity to disulphide bond-reducing agents. Immunoglobulins of one class have sedimentation rates of 7S and the second class of immunoglobulins have sedimentation rates of 19S. The latter are more sensitive to disulphide bond-reducing compounds, dissociating into smaller subunits and losing the capacity to combine with antigen after such treatment. The same procedure is ineffective with antibodies of the 7S class and the two classes can therefore be easily differentiated by 2-mercaptoethanol treatment.

We have made a study of the sulphydryl sensitivity of syphilitic antibodies, and have also investigated the temperature dependence of 2-mercaptoethanol treated and untreated sera in complement fixation, to determine whether they fix complement more efficiently at 4°C. or at 37°C. Cuniff and Stollar (1968) established with several antigen-antibody systems the temperature dependence of complement-fixing capacity. They found that 7S antibodies reacted much more efficiently at 37°C. than at 4°C. and that 19S antibodies fix complement better at 37°C. We have tried to discover how far the observations of Cuniff

and Stollar (1968) could be applied to syphilitic sera, and hence to decide whether complement fixation of syphilitic sera with warm fixation could be applied in daily routine work because the procedure takes much less time.

Material and methods

SERA

Specimens sent to our Institute for serological tests for syphilis were investigated by the VDRL and complement-fixation tests. Samples which fixed complement at a dilution of 1:4 or more were selected and stored at -20°C. until used. The 79 reactive sera studied by 2-mercaptoethanol treatment could be divided into three groups according to the available clinical data: eight sera originated from cases of primary syphilis, 37 from cases of secondary syphilis, and 34 from cases of latent or late syphilis. Among a further 184 reactive sera examined simultaneously in cold and in warm fixation, 101 were from patients with primary or secondary syphilis and 83 from patients with late syphilis.

ANTIGEN

A commercial product of Kolmer cardiolipin antigen was used. It was controlled by the 50 per cent. haemolysis method in our department (Surján and Füst, 1968a).

2-mercaptoethanol (ME) treatment

Two different buffer solutions for diluting sera were made up:

(1) Phosphate buffered saline, pH 7.2, alone; 0.2 M 2-ME in phosphate saline.

(2) Serum dilutions of 1:2 with and without 2-ME were allowed to set at room temperature for 4 hrs. The 2-ME was removed by extensive dialysis against the same buffered saline.

SERUM FRACTIONATION

Some representative serum samples were fractionated on Sephadex G-200 columns. Sephadex fraction I contained 19S antibodies and fraction II contained 7S antibodies. The fractions were not anticomplementary.

QUANTITATIVE COMPLEMENT-FIXATION TESTS

These were performed with a microtechnique using Takátsy's microtitrator (Takátsy, 1955). We had previously compared this micromethod and the standard

Kolmer technique on 1800 sera. The titres of 195 reactive sera obtained by parallel investigation with these two methods were estimated to \log_{10} -titres and results were mathematically analysed with Student's 't' test. The difference of the mean of \log_{10} -titres was not significant ($P > 0.3$) (Surján and Füst, 1968b).

The test itself was carried out by adding a calibrated drop of buffered saline (0.025 ml.) in the holes of the plastic plates; titrations were started from the original dilutions of 1:4 of serum, with the wire loop of the micro-titrator measuring 0.025 ml. serum dilutions into every hole. Antigen was diluted 1:75 and mixed with the same volume of complement dilution. From the antigen-complement mixture 0.025 ml. was dropped into the wells of the microtitrator and shaken vigorously. Reaction mixtures were incubated either at 4°C. for 18 hrs. or at 37°C. for 1 hr. before the addition of sensitized erythrocytes. It is to be noted that the amount of complement was varied for practical reasons so that four units of complement were employed in cold fixation and 1.5 units in warm fixation; this correction was necessary to diminish the difference between the warm and cold fixation. In both procedures 0.025 ml. of sensitized sheep erythrocyte suspension was dropped into every hole and the plates were incubated for 60 min. at 37°C. Thereafter the plates were put into a refrigerator for 15 min.

READING OF THE REACTION

The area of the pattern formed by the undissolved erythrocytes in the last dilution indicated the titres of the sera, which ranged between 16 and 1024.

STATISTICAL ANALYSIS

The titres of the complement-fixation tests were estimated to \log_{10} -titres according to the method of Kärber (1931). The results were evaluated by Student's 't' test.

Results

The complement fixation of 79 syphilitic sera and of their ME-treated samples was performed at 37°C. and at 4°C. simultaneously. The results are presented in the Table. ME treatment resulted in a highly significant ($P < 0.001$) titre decrease in both cold and warm fixation. Although we employed different amounts of complement so as to

diminish differences between cold and warm fixation, the titre decrease was more marked in warm fixation than in cold, and the difference was highly significant ($P < 0.01$).

According to sulphhydryl sensitivity and temperature dependence in complement fixation, the 79 reactive sera could be divided into five groups (Fig. 1).

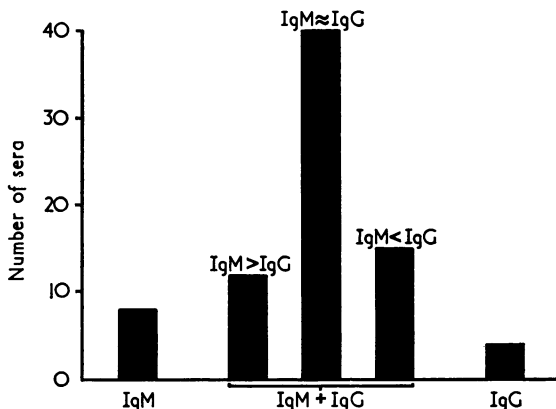


FIG. 1 *Immunoglobulin classes in 79 sera*

GROUP 1. SERA CONTAINING EXCLUSIVELY IgM

10 per cent. of the sera contained exclusively antibodies probably of IgM class. ME treatment effected a decrease to zero in the titre of the sera; the original titre was higher in warm fixation.

GROUP 2. SERA CONTAINING PREDOMINANTLY IgM

Before treatment the titres of the sera were higher in warm fixation; after treatment a significant fall in titre could be observed. The fact that some titres remained unaltered after ME treatment could be ascribed to the presence of antibodies of IgG class, which is why this residual titre is higher in cold fixation. 15 per cent. of sera belong to this category.

GROUP 3. SERA WITH IgM AND IgG ANTIBODIES IN APPROXIMATELY EQUAL PROPORTIONS

Half the sera (51 per cent.) were in this group. ME treat-

TABLE *Effect of 2-mercaptoethanol treatment on titre of complement-fixation reaction (comparison of cold and warm fixation)*

Sera	Fixation					
	Cold			Warm		
	No. of sera	Amount of \log_{10} -titres	Mean of \log_{10} -titres	No. of sera	Amount of \log_{10} -titres	Mean of \log_{10} -titres
Untreated	79	76.035	0.965	79	74.716	0.946
Treated	79	50.980	0.645	79	38.533	0.488
Decrease	—	25.055	0.320	—	36.183	0.458

B-A = 0.138**

** = highly significant difference ($P < 0.01$)

*** = highly significant difference ($P < 0.001$)

ment caused only a slight decrease in titre, and sera showed the same reactivity in cold and warm fixation.

GROUP 4. IgG PREDOMINANT IN 10 PER CENT.

Reactivity was higher in cold fixation before as well as after treatment; ME treatment caused only a slight change.

GROUP 5. SERA CONTAINING EXCLUSIVELY IgG

These formed 5 per cent. of the total. They were resistant to ME, and the reactivity was more effective in cold fixation.

Thus the majority of sera from syphilitic patients with primary, secondary, and late syphilis contained antibodies of both IgM and IgG classes. 10 per cent. of the sera contained exclusively IgM and only 5 per cent. exclusively IgG. This confirmed the supposition that complement fixation in syphilitic sera is temperature dependent; furthermore our findings that IgM occurs in or is predominant in the majority of the sera was also confirmed.

The question arises whether it is right to perform syphilitic complement-fixation tests at 4°C. for 18 hrs., if 19S antibodies are present in the majority of syphilitic sera examined. To resolve this question the complement fixation of 184 routine sera was carried out in parallel by cold and warm fixation. 84 per cent. of the 184 sera gave the same results in cold and warm fixation, 10 per cent. were more sensitive in cold, and 6 per cent. in warm (Fig. 2).

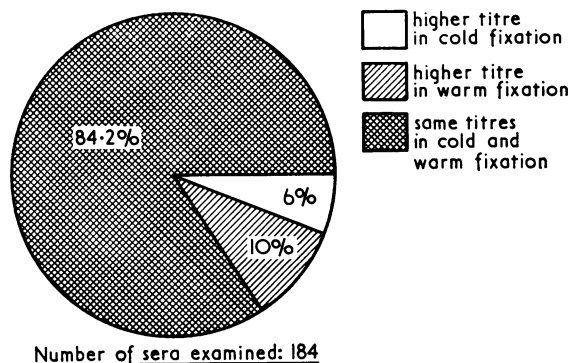


FIG. 2 Percentage results of cold and warm fixation in 184 sera

In Fig. 2 the titres are estimated to \log_{10} values. From the diagnostic point of view it is more important that out of the 184 sera four reacted only in cold fixation, and six only in warm fixation. This means that warm fixation can be safely used in our routine tests. It may also be stated that the use of the time-consuming cold fixation is not justified, because the 1 hr. incubation at 37°C. does not result in lower values. The practical significance of these findings is obvious.

The pattern of Sephadex fractions after ME treatment and their temperature dependence in complement fixation was the same as that of whole sera.

Discussion

The ME treatment of 79 syphilitic sera offered an opportunity to study the two most important immunoglobulin classes.

Cunniff and Stollar (1968) demonstrated in several antigen-antibody systems that not only the type of antigen but also the temperature has a marked influence on complement-fixation reactivity. 7S antibodies reacted more strongly at 4°C., while 19S antibodies reacted better at 37°C. It is of special interest that these authors obtained slightly different patterns just with syphilitic sera. Examining a pooled serum of high titre, they found that the whole serum reacted more strongly at 4°C. which could be ascribed to its higher IgG content. But the IgM fraction of this single serum did not react more effectively at 37°C. Our data demonstrate that the findings of Cunniff and Stollar (1968) observed in several antigen-antibody systems may also be valid in respect of the complement fixation of syphilitic sera.

Immunoglobulin sequence is one of the several unsolved problems concerning immunity against syphilis. It has been demonstrated in experimental syphilis that rabbit sera in the early stage of the disease contain anti-cardiolipin antibody of IgM type, while at later stages IgM and IgG types occur together. In late syphilitic rabbit sera only the reactivity of IgG antibodies could be detected (Julian and others, 1969). In human sera this sequence could not be demonstrated (Aho, 1967; Laurell and Malmquist, 1961). Our examinations cannot resolve this problem, but it must be stressed that in some sera we found exclusively IgM antibodies, and in others exclusively IgG antibodies among early as well as late syphilitic sera (Tringali, 1965).

Examination of the 184 routine sera also revealed that 73 per cent. of the sera, reacting more strongly in warm fixation (supposedly IgM dominance) originated from patients with early syphilis. On the other hand, 67 per cent. of the sera reacting more strongly in cold fixation were from patients with late syphilis. Thus IgM seems to prevail in recent infections, and IgG in the later stages, but the regular IgM-IgG sequence observed in other systems could not be detected in our specimens. Other reports in the literature also indicate that a regular IgM-IgG sequence cannot be regarded as a general phenomenon (Freeman and Stavitsky, 1965).

Summary

79 sera reactive in serological tests for syphilis were treated with 2-mercaptoethanol. According to the sulphydryl sensitivity the sera could be divided into five groups:

- (1) 10 per cent. contained exclusively antibodies of IgM class.
- (2) 15 per cent. showed IgM predominance.
- (3) 51 per cent. contained IgM and IgG antibodies in approximately equal proportions.
- (4) 19 per cent. showed IgG predominance.
- (5) 5 per cent. contained IgG alone.

The complement-fixation reactivity of antibodies of IgM class was more effective at 37°C. Complement-fixation reaction of 184 syphilitic sera examined in parallel by cold and warm fixation demonstrated that time-consuming cold fixation is not justified, because the 1-hr incubation at 37°C. did not result in lower titres.

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La sensibilité sulfhydrylique des anticorps syphilitiques et l'influence de la température dans la fixation du complément

SOMMAIRE

79 sérums sérologiquement réactifs pour la syphilis furent traités par le 2-mercatoethanol. Selon la sensibilité sulfhydrylique, les sérums peuvent être divisés en cinq groupes:

- (1) 10 pour cent contenaient exclusivement des anticorps du groupe IgM
- (2) 15 pour cent montraient une prédominance IgM
- (3) 51 pour cent contenaient des anticorps IgM et IgG en proportion à peu près égale
- (4) 19 pour cent montraient une prédominance IgG
- (5) 5 pour cent contenaient seulement des anticorps du groupe IgG

En réaction de fixation du complément, la réactivité des anticorps du groupe IgM a été plus grande à 37°C. L'étude de la réaction de fixation du complément de 184 sérums syphilitiques, examinés en parallèle après fixation à froid et à chaud, a montré que la fixation à froid, qui prend du temps, n'est pas justifiée car l'incubation pendant 1 heure à 37°C. ne donne pas des titres inférieurs.